CLAIMS

We claim:

1. A nucleic acid expression construct comprising a promoter operably linked to a polynucleotide encoding a mitochondrial permeability transition pore component polypeptide fused to an energy transfer molecule polypeptide, or a variant thereof.

2. The expression construct of claim 1 wherein the mitochondrial permeability transition pore component is an adenine nucleotide translocator.

3. The expression construct of claim 2 wherein the adenine nucleotide translocator is selected from the group consisting of human ANT1, human ANT2 and human ANT3.

4. The expression construct of claim 1 wherein the mitochondrial permeability transition pore component is selected from the group consisting of porin, hexokinase, creatine kinase, PRAX, CAML and the peripheral benzodiazepine receptor.

5. A nucleic acid expression construct comprising a promoter operably linked to a polynucleotide encoding a cyclophilin polypeptide fused to an energy transfer molecule polypeptide, or a variant thereof.

6. The expression construct of claim 5 wherein the cyclophilin is cyclophilin D.

7. The expression construct of claim 5 wherein the cyclophilin is selected from the group consisting of human cyclophilin A, cyclophilin B, human cyclophilin C and human Cyp-60.

8. A nucleic acid expression construct according to claim 1 or claim 5, wherein the construct comprises a vector selected from the group consisting of plasmids, cosmids, shuttle vectors, viral vectors and vectors comprising a chromosomal origin of replication.

9. A nucleic acid expression construct according to claim 8, wherein the vector comprises a plasmid selected from the group consisting of pBAD-His, pEYFP-C1, and pECFP-N1.

10. A nucleic acid expression construct according to claim 1 or claim 5 wherein the promoter is externally regulated.

11. A nucleic acid expression construct according to claim 1 or claim 5, wherein the energy transfer molecule is selected from the group consisting of a green fluorescent protein (GFP), a FLASH sequence and an aequorin protein.

12. The expression construct of claim 11 wherein the green fluorescent protein is selected from the group consisting of blue-shifted GFP, cyan-shifted GFP, red-shifted GFP and yellow-shifted GFP.

13. The expression construct of claim 11 wherein the energy transfer molecule is a derivative of an energy transfer molecule selected from the group consisting of a green fluorescent protein (GFP), a FLASH sequence and an aequorin protein.

14. A polypeptide comprising a mitochondrial permeability transition pore component polypeptide fused to an energy transfer molecule polypeptide, or a derivative thereof.

15. The polypeptide of claim 14 wherein the mitochondrial permeability transition pore component is an adenine nucleotide translocator.

16. The polypeptide of claim 15 wherein the adenine nucleotide translocator is selected from the group consisting of human ANT1, human ANT2 and human ANT3.

17. The polypeptide of claim 14 wherein the mitochondrial permeability transition pore component is selected from the group consisting of porin, hexokinase, creatine kinase, PRAX, CAML and the peripheral benzodiazepine receptor.

18. A polypeptide comprising a cyclophilin polypeptide fused to an energy transfer molecule polypeptide, or a derivative thereof.

19. The polypeptide of claim 18 wherein the cyclophilin is cyclophilin D.

20. The polypeptide of claim 18 wherein the cyclophilin is selected from the group consisting of human cyclophilin A, cyclophilin B, human cyclophilin C and human Cyp-60.

21. A polypeptide according to claim 14 or claim 18 wherein the energy transfer molecule is selected from the group consisting of a green fluorescent protein (GFP), a FLASH sequence and an aequorin protein.

22. A polypeptide according to claim 21 wherein the green fluorescent protein is selected from the group consisting of blue-shifted GFP, cyan-shifted GFP, red-shifted GFP and yellow-shifted GFP.

23. A host cell for identifying agents that alter mitochondrial permeability transition, comprising:

(a) a first nucleic acid expression construct, comprising a promoter operably linked to a polynucleotide encoding a mitochondrial permeability transition pore component

polypeptide fused to a polynucleotide encoding a first energy transfer molecule or a variant thereof; and

(b) a second nucleic acid expression construct, comprising a promoter operably linked to a polynucleotide encoding a cyclophilin polypeptide fused to a polynucleotide encoding a second energy transfer molecule or a variant thereof, wherein binding of the mitochondrial permeability transition pore component polypeptide to the cyclophilin polypeptide results in detectable energy transfer between the first and second energy transfer molecules.

24. The host cell of claim 23 wherein the mitochondrial permeability transition pore component is an adenine nucleotide translocator.

25. The host cell of claim 24 wherein the adenine nucleotide translocator is selected from the group consisting of human ANT1, human ANT2 and human ANT3.

26. The host cell of claim 23 wherein the mitochondrial permeability transition pore component is selected from the group consisting of porin, hexokinase, creatine kinase, PRAX, CAML and the peripheral benzodiazepine receptor.

27. The host cell of claim 23 wherein the cyclophilin is selected from the group consisting of human cyclophilin A, cyclophilin B, human cyclophilin C and human Cyp-60.

28. A host cell according to claim 23, wherein the host cell is a prokaryotic cell.

29. A host cell according to claim 23, wherein the host cell is a eukaryotic cell.

30. A host cell according to claim 39, wherein the eukaryotic cell is selected from the group consisting of 293, COS-7, Sf9, CHO, Hep-2, MDCK and Jurkat.

31. A host cell according to claim 23, wherein the first and second energy transfer molecules are selected from the group consisting of green fluorescent protein (GFP), blue-shifted GFP, cyan-shifted GFP, red-shifted GFP and yellow-shifted GFP.

32. A host cell according to claim 23, wherein the first and second energy transfer molecules have an excitation maximum at a wavelength ranging from 300 nm to 650 nm, and an emission maximum at a wavelength ranging from 350 nm to 675 nm.

33. A host cell according to claim 23, wherein the first energy transfer molecule and the second energy transfer molecule have excitation and emission maxima at different wavelengths.

34. A host cell according to claim 23, wherein at least one nucleic acid expression construct is extrachromosomal.

35. A host cell according to claim 27, wherein at least one nucleic acid expression construct is integrated into a host cell chromosome.

36. A host cell according to claim 35 wherein the host cell chromosome is a mitochondrial chromosome.

37. A method for screening for an agent that alters mitochondrial permeability transition (MPT), comprising the steps of:

(a) contacting a host cell according to claim 23 comprising a mitochondrion with a candidate agent and an inducer of MPT;

(b) exposing the cell to an excitation energy;

(c) detecting a level of energy transfer between the first and second energy transfer molecules; and

(d) comparing the level of energy transfer to a first reference level generated in the absence of candidate agent, and therefrom identifying an agent that alters MPT.

38. The method of claim 37, wherein the host cell is further contacted with an inhibitor of MPT to generate a second reference level.

39. The method of claim 38, wherein the inhibitor of MPT is selected from the group consisting of low pH, inducers of high mitochondrial membrane potential, and cyclosporin A.

40. A method according to claim 37, wherein the inducer of MPT is atractyloside or bonkrekic acid.

41. A method according to claim 37, wherein the inducer of MPT comprises a compound that increases Ca^{+2} concentration in the mitochondria.

42. A method according to claim 41, wherein the compound is selected from the group consisting of ionophores, ionomycin, thapsigargin, amino acid neurotransmitters, glutamate, N-methyl-D-aspartic acid, carbachol, apoptogens, and inducers of potassium depolarization.

43. A method according to claim 37, wherein the host cell is further contacted with an inducer of oxidative stress.

44. A method according to claim 43, wherein the inducer of oxidative stress is selected from the group consisting of ethacrynic acid, buthionine sulfoximine, diamide, menadione, *t*-butyl hydroperoxide, phenyl-arsine oxide and nitric oxide.

45. A method according to claim 37, wherein the candidate agent increases energy transfer between the first and second energy transfer molecules.

46. A method according to claim 37, wherein the candidate agent decreases energy transfer between the first and second energy transfer molecules.

47. A method according to claim 37, wherein the first and second energy transfer molecules are selected from the group consisting of green fluorescent protein (GFP), blue-shifted GFP, cyan-shifted GFP, red-shifted GFP and yellow-shifted GFP.

48. A method according to claim 37, wherein the excitation energy is light with a wavelength ranging from 300 nm to 650 nm.

49. A method according to claim 37, wherein the first and second energy transfer molecules have an excitation maximum at a wavelength ranging from 300 nm to 650 nm, and an emission maximum at a wavelength ranging from 350 nm to 675 nm.

50. A method according to claim 37, wherein the first energy transfer molecule and the second energy transfer molecule have excitation and emission maxima at different wavelengths.

51. A method according to claim 37, wherein:

(a) the first energy transfer molecule has an excitation maximum at a wavelength ranging from 400 nm to 500 nm and an emission maximum at a wavelength ranging from 450 nm to 525 nm, and the second energy transfer molecule has an excitation maximum at a wavelength ranging from 450 nm to 525 nm and an emission maximum at a wavelength ranging from 500 nm to 550 nm; or

(b) the second energy transfer molecule has an excitation maximum at a wavelength ranging from 400 nm to 450 nm and an emission maximum at a wavelength ranging

from 450 nm to 500 nm, and the first energy transfer molecule has an excitation maximum at a wavelength ranging from 500 nm to 525 nm and an emission maximum at a wavelength ranging from 525 nm to 550 nm.

52. A method according to claim 37, wherein:

(a) the first energy transfer moleeule has an excitation maximum at a wavelength of about 433 nm and an emission maximum at a wavelength of about 475 nm, and the second energy transfer molecule has an excitation maximum at a wavelength of about 513 nm and an emission maximum at a wavelength of about 527 nm; or

(b) the second energy transfer molecule has an excitation maximum at a wavelength of about 433 nm and an emission maximum at a wavelength of about 475 nm, and the first energy transfer molecule has an excitation maximum at a wavelength of about 513 nm and an emission maximum at a wavelength of about 527 nm.

53. A method for detecting an agent that alters mitochondrial permeability transition (MPT), comprising the steps of:

(a) contacting a cyclophilin D polypeptide with an adenine nucleotide translocator polypeptide and a candidate agent, under conditions and for a time sufficient to permit the cyclophilin D, adenine nucleotide translocator, and the candidate agent to interact; and

(b) detecting a level of binding of cyclophilin D polypeptide to adenine nucleotide translocator polypeptide, relative to a level of binding detected in the absence of the candidate agent, and therefrom detecting an agent that alters MPT.

54. A method according to claim 53, wherein the cyclophilin D polypeptide is immobilized on a support.

55. A method according to claim 53 or claim 54, wherein the cyclophilin D polypeptide is a fusion protein.

56. A method according to claim 53, wherein the adenine nucleotide translocator polypeptide is immobilized on a support.

57. A method according to claim 53 or claim 56, wherein the adenine nucleotide translocator polypeptide is a fusion protein.

58. A method according to claim 55 or claim 57, wherein the fusion protein comprises a protease recognition sequence.

59. A method according to claim 55 or claim 57, wherein the fusion protein comprises a ligand for a receptor.

60. A method according to claim 53, wherein the candidate agent is selected from the group consisting of peptides, polypeptides, proteins and small molecules.

61. A method according to claim 53, wherein the candidate agent is a small molecule present within a combinatorial library.

62. An agent capable of altering mitochondrial permeability transition, wherein the agent is identified by a method of claim 37 or claim 53.

63. A method for altering survival of a cell, comprising contacting a cell with an agent identified according to claim 37 or claim 53, under conditions and for a time sufficient to modulate cell survival.

64. A method for altering mitochondrial permeability transition (MPT), comprising contacting a mitochondrion with an agent identified according to claim 37 or claim 53, under conditions and for a time sufficient to alter MPT.

65. A method according to elaim 64, wherein the mitochondrion is present within a cell.

66. The method of claim 65, wherein the cell is present within a living organism.

67. The method of claim 65, wherein the cell is a cybrid cell.

68. A method for preparing a mitochondrial permeability transition pore component polypeptide fused to an energy transfer molecule, comprising the steps of:

(a) culturing a host cell comprising a nucleic acid expression construct that encodes a fusion protein comprising an adenine nucleotide translocator polypeptide or a derivative thereof fused to an energy transfer molecule polypeptide or a derivative thereof, under conditions that permit expression of the fusion protein; and

(b) recovering fusion protein from the culture.

69. The method of claim 68 wherein the mitochondrial permeability transition pore component is an adenine nucleotide translocator.

70. The method of claim 69 wherein the adenine nucleotide translocator is selected from the group consisting of human ANT1, human ANT2 and human ANT3.

71. The method of claim 68 wherein the mitochondrial permeability transition pore component is selected from the group consisting of porin, hexokinase, creatine kinase, PRAX, CAML and the peripheral benzodiazepine receptor.

72. A method for preparing a cyclophilin polypeptide fused to an energy transfer molecule, comprising the steps of:

(a) culturing a host cell comprising a nucleic acid expression construct that encodes a fusion protein comprising a cyclophilin polypeptide or a derivative thereof fused to an energy transfer molecule polypeptide or a derivative thereof, under conditions that permit expression of the fusion protein; and

(b) recovering fusion protein from the culture.

73. The method of claim 72 wherein the cyclophilin polypeptide is a cyclophilin D polypeptide.

74. The method of claim 72 wherein the cyclophilin polypeptide is selected from the group consisting of human cyclophilin A, cyclophilin B, human cyclophilin C and human Cyp-60.

75. A method according to claim 68 or claim 72, where the host cell is a prokaryotic cell.

76. A method according to claim 68 or claim 72, wherein the host cell is a eukaryotic cell.

77. The method of claim 76, wherein the eukaryotic cell is selected from the group consisting of 293, COS-1, Sf9, CHO, Hep-2, MDCK and Jurkat.

78. A method according to claim 68 or claim 72, wherein the nucleic acid expression construct is extrachromosomal.

79. A method according to claim 68 or claim 72, wherein the nucleic acid expression construct is integrated into a host cell chromosome.

80. The method of claim 79 wherein the host cell chromosome is a mitochondrial chromosome.

81. A method according to claim 68 or claim 72, wherein the fusion protein comprises a recognition sequence for a protease.

82. A method according to claim 68 or claim 72, wherein the fusion protein comprises a ligand for a receptor.

83. A kit for screening for agents that alter mitochondrial permeability transition, comprising:

(a) an isolated cyclophilin D polypeptide or a derivative thereof;

(b) an isolated adenine nucleotide translocator polypeptide or a derivative thereof; and

(c) a detection reagent that specifically binds to at least one of the foregoing polypeptides.

84. A kit according to claim 83, wherein the cyclophilin D polypeptide is immobilized on a support.

85. A kit according to claim 83, wherein the adenine nucleotide translocator polypeptide is immobilized on a support.

86. A kit according to claim 83, wherein the detection reagent is an antibody or antigen-binding fragment thereof.

87. A kit for screening for agents that alter mitochondrial permeability transition (MPT), comprising:

(a) a host cell;

(b) a first nucleic acid expression construct, comprising a promoter operably linked to a polynucleotide encoding an adenine nucleotide translocator polypeptide fused to a first energy transfer molecule or a variant thereof; and

(c) a second nucleic acid expression construct, comprising a promoter operably linked to a polynucleotide encoding a cyclophilin D polypeptide fused to a second energy transfer molecule or a variant thereof.

88. A kit according to claim 87, wherein the host cell is a prokaryotic cell.

89. A kit according to claim 87, wherein the host cell is a eukaryotic cell.

90. The kit of claim 89, wherein the eukaryotic cell is selected from the group consisting of 293, COS-1, Sf9, CHO, Hep-2, MDCK and Jurkat.

91. A kit according to claim 87, wherein the first and second energy transfer molecules are selected from the group consisting of green fluorescent protein (GFP), blue-shifted GFP, cyan-shifted GFP, red-shifted GFP and yellow-shifted GFP.